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(57) Abstract

A stanol composition containing in addition to sitostanol as the main component, also a substantial amount of at least 10 % campestanol has been found to effectively lower serum cholesterol levels when incorporated in edible commodities. Upon esterification the composition is especially useful in edible fats and oils and in fat-containing foods.

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STANOL COMPOSITION AND THE USE THEREOF

Field of the Invention

The present invention relates to a sitostanol containing composition of plant stanols especially for use as a serum cholesterol level lowering substance. The invention also relates to the corresponding esterified form of such a composition which advantagously can be used in edible oils and fats and in fat-containing foods.

Background of the Invention

Plant sterols are essential components of all plants. Their functions in plants resemble the functions of cholesterol in mammals. The most abundant plant sterols in the flora are ß-sitosterol, campesterol and stigmasterol. The chemical structure of these plant sterols is very similar to that of cholesterol the differences occuring in the side chain of the backbone of the molecule. For example, compared to cholesterol, the side chain of sitosterol contains an additional ethyl group and the side chain of campesterol an additional methyl group.

Since 1950's plant sterols have been known to effectively reduce the serum cholesterol levels. Even when administered in relatively small doses (a few grams a day) they reduce the absorbability of both biliary and dietary cholesterol effectively and thus lower the serum total and LDL-cholesterol levels (12, 28, see also 27, 32). The mechanism by which the restriction of cholesterol absorption happens is still not known in detail, but it is assumed that plant sterols displace cholesterol from the micellar phase and thereby prevent its absorption. In practically all of the early studies, sitosterol or its hydrogenated form sitostanol has been the main plant sterol of interest. However, the sterol composition of the tested preparations has not always been well documented, and the sterol preparations used in most studies have also contained different amounts of other sterols.

Plant sterols have been considered as a saf way of lowering serum cholesterol levels, since they are natural components of vegetable fats and oils. Additionally, their absorption from the intestine of healthy subjects is limited, and th limited

amounts absorbed are excreted from the body in the bile. The absorbtion rate of the plant sterols varies between individuals and between the different plant sterols, but for healthy humans usually less than 5% of the plant sterols are absorbed from the digestive tract (27). However, up to 10% of dietary campesterol has been shown to be absorbed (20).

In few rare diseases such as sitosterolemia plant sterols are absorbed exceptionally efficiently, and also the elimination from the body via the biliary route is impaired. Serum levels of sitosterol, campesterol and also their saturated forms sitostanol and campestanol are highly elevated. The elevated levels of the saturated stanols are most probably due to their more effective endogenous synthesis rather than a more effective absorption (10, 27). If untreated, sitosterolemia leads already at young age to xanthomatosis and coronary heart disease. For people with this disease, an administration of unsaturated plant sterols in amounts greater than normally present in foods may lead to hazardous health effects.

Lees and Lees (25) tested the effects of three different sitosterol preparations on plasma lipid and lipoprotein concentrations. One of the preparations was Cytellin, a commercial preparation (Eli Lilly Co., USA) that contained 60-65% sitosterol and 35-40% other sterols, mainly campesterol. An average dose of 18 g/day divided in three doses resulted in a 10.5% average fall in plasma total cholesterol and a 15% fall in LDL-cholesterol. However, when only traces of plant sterols including campesterol are normally detected in plasma (10, 33), the plasma concentration of campesterols varied from 4 to 21 mg/dl in the subjects tested by Lees and Lees (25). In the discussion the authors stated very strongly that since the atherogenicity of campesterol is unknown, the use of a sitosterol preparation with a relatively high campesterol content like the Cytellin preparation used in their study cannot be recommended.

Further, Lees et al. (26) studied the efficacy of plant sterols from soybean oil and tall oil in lowering the blood cholesterol level. They used two different physical forms of each plant sterol, namely a suspension and a powder. The soy sterol consisted of 60-65% sitosterol and 35% campesterol, and a daily dose of an average 18 g of sterols per day (range 9-24 g) was given in three equal doses. A tall oil sterol preparation with only about 5% campesterol was used in this study. A daily dose of 3 grams of both tall oil sterol preparations (powder and suspension)

was tested. Additionally, a dose of 6 grams of the tall oil sterol suspension was tested.

Soy sterol in both physical forms and tall oil sterol in powder form reduced the plasma cholesterol content by on average 12% (26). However, the relatively high absorbability of campesterol that has already been shown earlier, was observed also in this study. In the 5 patients tested the plasma campesterol levels ranged from 5 to 21 mg/dl (mean 16 mg/dl). Thus again, even if the cholesterol-lowering effect of soy sterol was proved to be significant, the authors did not recommend its use as a cholesterol-lowering agent. On the contrary, they recommended that pharmaceutical plant sterol preparations should contain a minimum of campesterol and a maximum of sitosterol. Based on the two studies cited above, it can be concluded that the use of vegetable oil based sterols such as soy sterol are strongly not recommendable.

Saturated plant sterols such as sitostanol and campestanol are present in most vegetable oils only in trace amounts. However, tall oil sterols contain 10-15% of sitostanol, the saturated form of sitosterol. Sitostanol can also be made by hydrogenation of the double bond in sitosterol. In the latest studies made with both experimental animals and humans, sitostanol has been proven to be more effective as a cholesterol-lowering agent than sitosterol (8, 16, 17, 18, 19, 36).

An additional advantage of sitostanol is that it is virtually unabsorbable. Several studies (e.g. 9, 16, 17, 21) have shown that sitostanol is practically unabsorbable while small amounts (<5%) of its unsaturated form sitosterol (33) can be absorbed. Similarily, in an *in vitro* study Amstrong and Carey (6) also showed that cholestanol, a saturated form of cholesterol, was more hydrophobic and less absorbable than cholesterol.

When sitostanol is made by hydrogenation of the most usual plant sterol sources, also another saturated plant sterol, namely campestanol, is formed from campesterol. Until recently, relatively little has been known about the absorbability and the possible hypocholesterolemic effect of this stanol. Based on the data cited above stating that saturated sterols are less absorbable than their unsaturated forms, it could be hypothesized that campestanol might be virtually unabsorbable.

To study the absorbability of different plant sterols Heinemann et al. (20) compared the intestinal absorption of cholesterol with campesterol, sitosterol, stigmasterol and also low concentrations of sitostanol and campestanol in humans by means of intestinal perfusion technique. The results showed that the absorption rate of the differed plant sterols varied between different plant sterols being on average 4.2% for sitosterol, 4.8% for stigmasterol, 9.6% for campesterol and 12.5% for campestanol. Large variation between the absorption efficacy in the ten male subjects was detected.

Thus, according to Heinemann et al. (20) campestanol was found to be more efficiently absorbed than its unsaturated form campesterol. This is against the assumption based on studies cited earlier that showed that the saturated sterols (sitostanol, cholestanol) would be less absorbable than the unsaturated ones (sitosterol, cholesterol). The reason for this remains unclarified. Heinemann et al. (20) speculated, though, that the reason for this conflicting result might be that the study of Amstrong and Carey (6) was made with *in vitro* conditions and that the theory of the hydrophobicity being a major factor in micellar binding and/or absorption might not be relevant in *in vivo* conditions. However, this speculation does not explain the fact that several studies that have shown the poorer absorbability of sitostanol compared to that of sitosterol have been made under *in vivo* conditions. Thus the results of Heinemann et al. (20) that conflict with previous results remained unexplained by the authors.

Sugano et al. (34) studied the hypocholesterolemic activity of corn sterols (composition: 31% campesterol, 4% stigmasterol and 65% sitosterol) and corn stanols (composition: 31% campestanol and 69% sitostanol) obtained by hydrogenation of a corn oil sterol mixture. Two experiments were carried out in rats. Both the sterol and the stanol showed hypocholesterolemic effects at the level of 0.5-1% of the diet when cholesterol (1% in the diet) was ingested. In the first experiment no significant difference was sen in the hypocholesterolemic effect of phytosterols and phytostanols. However, in the second experiment, at the same dietary levels the phytostanols showed considerably greater ability to lower the plasma cholesterol concentration tham did the phytosterols (statistically significant at $p<0.02$). Moreover, rats fed the 1.0% stanol diet had plasma chol sterol levels significantly lower ($p<0,02$) than that of the animals fed the diet free of cholesterol. This was not observed in rats fed the 1.0% sterol diet.

Sugano et al. (34) did not study the difference in hypocholesterolemic effect between stanol mixtures with a high content of sitostanol and a low content of campestanol (tall oil sterol based) and stanol mixtures with a substantially higher level of campestanol (vegetable oil sterol based). They compared the hypocholesterolemic effect of an unsaturated sterol mixture with the corresponding saturated stanol mixture. Later studies made by this research group have been focused on the cholesterol lowering effect of sitostanol specificly and compared to sitosterol (21, 22, 23, 35). In fact, in a later publication (23) they refer to the phytostanol study mentioned above (34) mentioning only the hypocholesterolemic effect of β-sitostanol compared to β-sitosterol without discussing any hypocholesterolemic effect of saturated sterols (including campestanol) compared to unsaturated sterols. In the later studies mentioned above sterol mixtures with the typical composition of hydrogenated tall oil sterols with a high content of sitostanol (>90%) have been used.

Miettinen and Vanhanen (30) have shown that sitostanol in fatty acid ester form is more effective than free sitostanol in lowering serum cholesterol levels. Later studies have also shown that the use of sitostanol esters as a part of a daily diet is an effective way of reducing serum total and LDL-cholesterol concentrations (13, 14, 15, 31, 37, 38). The benefit of using stanol esters instead of free stanol is also that the stanol esters are fat-soluble and can therefore easily be incorporated into a wide variety of foods without changing the taste, flavor or physical behavior of the final product. The method for the preparation of sitostanol fatty acid esters and the use of fat-soluble stanol esters in foods have been disclosed in US Patent No 5,502,045 (2), hereby incorporated by reference.

Straub (3) suggests the use of saturated stanols (sitostanol, clionastanol, 22,23-dihydrobrassicastanol, campestanol and mixtures thereof) in a method for making a food additive composition where stanols are mixed with an edible solubility agent, an effective amount of a suitable antioxidant and an effective amount of a suitable dispersant. These food additives are intended to reduce cholesterol absorption from foods and beverages which contain cholesterol, e.g. meat, eggs and dairy products. However, in this patent no data showing either any clinical effects or the absorbtion of dietary sterols is presented.

Eugster et al. (1) teach the use of small amounts of sterols, their fatty acid esters and glucosides for the treatment of tumors. The methods of preparation proposed by Eugster et al. involve hazardous chemical reagents like N,N'-carbonyl-

diimidazole, thionyl chloride and solvents like tetrahydrofuran, benzen, chloroform or dimethylformamide. Eugster et al. comment on the possible use of these substances as dietary foods and as food additives, but do not present any data on hypocholesterolemic effects or make any claims covering such use. From the disclosure of Eugster et al. it is hard to get a clear picture of how the end product is purified to yield a pure enough sterol ester in large amounts enough to be used as a food component. The only purifying processes referred to are thin layer chromatography and high performance liquid chromathoghaphy. This being the case, the preparation method referred to in the patent by Eugster et al. is limited to small amounts only.

The US patent 3,751,569 (4) discloses the addition of plant sterol fatty acid esters to cooking oil with the objective of lowering the serum cholesterol levels in man. The patent proposes, for use in the esterification of free sterols, a method which in no case fullfills the requirement for preparation of a food-grade product. According to the patent, the esterification is carried out between a free sterol and a fatty acid anhydride, with perchloric acid acting as a catalyst. The catalyst and reagent used cannot be accepted in food processes. In addition, the patent relates to the fatty acid esters of only native plant sterols. The method proposed in the German patent DE 22 48 921 (5) for the esterification of sterols present in oils and fats by a chemical interesterification technique fullfills the criteria of food processes. In this patent, free sterol and an excess of fatty acid esters are added to a mixture of oil or fat, whereafter the entire fat blend is interesterified by a commonly known interesterification technique. In the resulting fat blend virtually all free sterols have been converted to fatty acid esters.The purpose of this is to protect free sterols in vegetable and animal oils against possible changes during processing.

Earlier data shows that campesterol, one of the major plant sterols, is absorbed relatively efficiently. Therefore it has been recommended that only plant sterol mixtures with a minimum content of campesterol should be used. This has in practice lead to the use of sterol mixtures such as tall oil sterols with a high content of sitosterol.

Most work on stanols has cover d sitostanol only. The study of Heinemann et al. (20) sh wing that campestanol, the saturated form of campesterol, is more readily absorbed than campesterol or sitosterol (12.5%, 9.6% and 4.2% respectively) has lead to a "consensus" that saturated sterol mixtures with "elevated" levels of

campestanol ar unsafe due to the absorption of campestanol. A clear evidence of this is that all clinical studies covering the use of stanols (sitostanol) have been based on sterol mixtures with a high level of sitostanol and a low level of campestanol.

It is an established fact from many studies (e.g. 8, 17, 18, 19, 23, 36), that sitostanol, the saturated form of sitosterol, is more effective than the corresponding unsaturated sitosterol in reducing the blood cholesterol level. Furthermore saturated sterols are absorbed in very limited amounts, which make the use of saturated sterols a safe mean of reducing cholesterol on a population bases. Of the unsaturated sterols especially campesterol is absorbed in amounts high enough to call for strong recommendations against the use of sterol mixtures with eleveted levels of campesterol (eg. vegetable oil based sterol mixtures) (25, 26).

Accordingly there has been a strong prejudice against using campestanol in any substantial amounts as a substance to be added to foods and this has seriously limited the spectrum of phytosterol containing raw materials to such containing a relatively minor amount of campesterol and its saturated form, campestanol.

Brief Description of the Invention

This invention relates to plant stanol compositions containing sitostanol as a main component but with substantial amounts of campestanol, either in free form or esterified as fatty acid esters for lowering the level of blood serum cholesterol.

The invention further relates to the use of stanol compositions containing sitostanol as the main component but also substantial amounts of campestanol, or fatty acid esters thereof in edible commodities as a dietary component for lowering blood serum cholesterol levels.

The object of the present invention is to broaden the spectrum of plant raw materials useful in the preparation of substances for edible commodities, especially edible oils and fats and fat-containing foods intended to control chol st rol levels in blood serum. The invention enables using as raw materials for these purpos s plant oils and fats containing in addition to sitosterol also a substantial amount of campesterol.

Suitable raw materials for use in the preparation of the compositions of the present invention are e.g. corn, soybean and rapeseed but also other plants with a phytosterol composition high in campesterol may be used.

The novel composition of the present invention, and especially its esterified form, may be incorporated in food substances such as cooking oils, margarines, butter, mayonnaise, salad dressings, shortenings, cheeses (including unripened and ripened cheeses) and other fat-containing foods.

The composition of the present invention can also be consumed as such.

Detailed Description of the Invention

According to the present invention, the plant stanol composition contains, in addition to its main component, sitostanol, also a substantial amount of at least 10% campestanol.

The composition preferably contains as much as from 20% to 40% and most preferably from 25% to 35%, e.g. about 30% campestanol or its fatty acid ester when the composition has been esterified to make it lipophilic.

Throughout this specification all percentages are given by weight, unless otherwise specified. In this specification the bracketed numbers refer to publications listed in the appended List of References.

Data obtained surprisingly and against prevailing prejudice shows that a hydrogenated stanol mixture containing sitostanol as the main component but with substantial amounts of campestanol is at least as effective as a stanol mixture containing over 90% sitostanol and a low level of campestanol, indicating that campestanol is at least as effective in reducing the absorption of cholesterol as sitostanol. Moreover, data from sterol analysis of blood serum clearly shows that campestanol remains virtually unabsorbed, with blood serum contents being about 40% smaller than that of sitostanol. Thus a stanol mixture containing sitostanol as a major component but with substantial amounts of campestanol must be regarded as at least as safe as a conventional tall sterol based stanol mixture. This data is in striking contrast to current opinion regarding the efficacy and safety of stanol mixtures with elevated amounts of campestanol (see 20, 27, 34).

The US Patent No 5,502,045 (2) showed that fatty acid esters of sitostanol are more effective in reducing the blood cholesterol level than the free sitostanol. Later studies have clearly confirmed the cholesterol lowering effect of a margarine containing fat soluble sitostanol fatty acid esters (e.g. 31).

The use of stanol fatty acid esters instead of free stanols is crucial for a broad use of these in various fat containing food products because only the stanol fatty acid esters are soluble in edible oils and fats in amounts high enough to reach levels effective in reducing the absorption of both dietary and biliary cholesterol from the digestive tract.

The solubility of stanol esters in edible oils and fats is as high as 35-40%, whereas the solubility of free sterols in edible oils and fats is limited to a maximum of 2 per cent by weight only at the temperature of 21°C (24). Higher amounts could be incorporated by using different surfactancts, solubilizing or dispersing agents, but even the use of these substances does not ensure fat solubility. The use of the above substances is usually restricted or even prohibited by law. Furthermore free sterols at a level of 1% will affect the physical properties of the fat or oil, causing changes in the structure and physical behaviour of the product. This is not the case when stanol fatty acid esters are used since the physical properties of the fat mixture can easily be modified by altering the fatty acid composition of the mixture.

It is obvious that stanol fatty acid esters easily can be incorporated to other foods than margarines and spreads as described in this invention. The US Patent No 5,502,045 (2) gives further examples of possible use. It is, however, obvious to those skilled in the art that stanol fatty acid esters can be added to a wide variety of foods, especially fat-containing foods.

Many methods for preparing fatty acid esters of sterols have been proposed. The drawbacks of these methods are that almost all of them use reagents, which cannot be accepted in the production of a product intended to be used as a macronutrient in foods. The use of toxic reagents like thionyl chloride or anhydride derivatives of fatty acids is common.

The preferred method of preparing stanol fatty acid sters of sterols is described in the US patent No 5,502,045 (2, hereby incorporated by reference). This procedur is based on the interesterification process used widely by the edible fat

and oil industry. This esterification process deviates advantageously from previous methods in that no other substances than the free stanol, a fatty acid ester or a fatty acid ester mixture and a interesterification catalyst like sodium ethylate are used. One important feature of the method is that one of the reactants, the fatty acid ester is used in excess and functions as a solvent, solubilizing the stanol under the conditions used (vacuum 5-15 mmHg). The reaction gives a mixture of fatty acid esters and stanol fatty acid esters. The stanol fatty acid ester can easily be concentrated into almost pure stanol fatty acid esters by vacuum destillation, which removes the excess of fatty acid esters. Alternatively the blend can be added as such to the final fat blend before the deodorizing step is carried out.

Stanols are found in small amounts in nature eg. in wheat, rye, corn and tritricale and can thus be found in small amounts (11, 14) in the daily food. Stanols can easily be produced by hydrogenation of natural sterol mixtures. Only tall sterol mixtures with high enough purity (sterol content >98%) to be used as such for food use were commercially available in early 1996. Plant sterols with substantial amounts of campesterol such as vegetable oil based sterol mixtures can e.g. be obtained as a by-product of tocopherol production from vegetable oil distillates. The obtained plant sterols can be converted into stanols by prior known hydrogenation techniques such as that based on the use of Pd/C catalyst in organic solvents (7, hereby incorporated by reference). It is obvious for those skilled in the art that a wide variety of Pd catalysts and solvents can be used to carry out the hydrogenation, which when done under optimized conditions leaves only small amounts of unsaturated sterols unconverted while the formation of the typical dehydroxylated by-products stanes and stenes remains at a low level (<1.5%).

The instant invention compares the hypocholestelolemic effect of a stanol mixture containing a high level of sitostanol that is generally regarded by experts in the field to be the safest and most effective plant sterol in reducing cholesterol absorption and thereby serum cholesterol levels with a stanol mixture containing a substantial amount of campestanol. In this specification, for the first time, hypocholesterolemic effects of vegetable oil based stanols in humans have been reported. This invention is the first to show that a stanol mixture with a substantial amount of campestanol (over 10% and preferably about 30 %) is at least as effective as stanol mixtures with high levels of sitostanol. Furthermore, the results

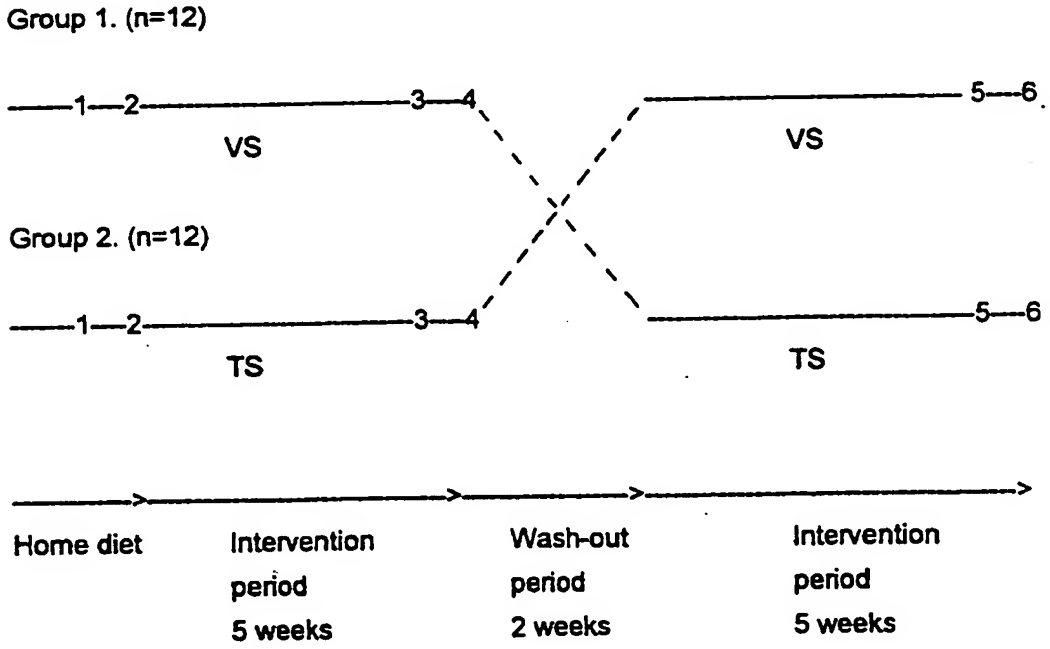
of the present study clearly indicate that camp stanol on the contrary to what has been reported by Heinemann et al. (20) is virtually unabsorbed.

Clinical Studies

To study the hypocholesterolemic effects of vegetable oil stanol ester and tall oil stanol ester margarines a 5-week double blind cross over study with a 2 weeks wash-out period was designed. The test arrangement of the study was as follows:

Test arrangement of the intervention study.

Numbers 1-6 indicate the blood samples collected at the home diet (1, 2), after the first intervention period (3, 4) and after the second intervention period (5, 6). VS = vegetable oil based stanol ester margarine, TS = tall oil based stanol ester margarine.



Twenty-four voluntary, free-living, healthy women with a moderately elevated cholest rol l v l (average 6.12±0.16 mmol/l) consumed about 25 g per day (a 250 g tub/ 10 days) of the t st margarines as a part of the daily diet in a random order. S rum lipids (total chol sterol, LDL-chol sterol, HDL-cholest rol and tri-glycerides) and s rum sterol contents were m asured at the hom diet and at the end of each test period. Blood samples were taken twice, one w ek apart at the

home diet and by the end of each test margarine perios.The obtained serum lipid values are shown in Table 1 below.

Table 1. Serum lipid concentrations (mmol/l, mean±SE) during the home diet and after the five-week treatment with vegetable oil stanol ester margarine (VS) and tall oil stanol ester margarine (TS), (n=24).

	Home diet	VS	TS
Total cholesterol	6.12±0.16	5.77±0.18*	5.95±0.23
LDL-cholesterol	4.03±0.15	3.60±0.17*	3.76±0.19*
HDL-cholesterol	1.54±0.09	1.62±0.09*	1.63±0.10*
Triglycerides	1.22±0.13	1.20±0.11	1.26±0.15

* $p<0.05$ or less

Both test margarines resulted in favourable changes in serum lipids. The reduction in LDL-cholesterol values and the increase in HDL-cholesterol values were statistically significant ($p<0.05$ or less). Furthermore, the vegetable oil based sterol ester resulted also in a statistically significant reduction of total cholesterol. The obtained reduction of total cholesterol and LDL-cholesterol was higher with the vegetable oil based stanol ester margarine compared to the tall oil based stanol ester margarine. No changes in triglyceride levels were obtained. The serum lipid results obtained indicate that a vegetable oil stanol ester margarine containing a substantial amount of campestanol in its stanol fraction might be even more effective than the tall oil stanol ester margarine. Tall oil stanol ester margarine has in earlier studies (14, 15, 31) shown effective hypocholesterolemic effects. Thus, based on the cross-over design of this study, it can be concluded that vegetable oil based stanols are showing at least as effective hypocholesterolemic effects as tall oil based stanols.

Serum sterol concentrations were quantified with gas-liquid cromatography according to a previously published method (29, hereby incorporated by reference). The means of two m asur ments of serum lipids from the blood samples taken at each period were calculated. The data on mean serum plant sterol concentrations at the home diet and after each test period and the mean changes observed in these concentrations are presented in Tables 2 and 3 below.

Table 2. Serum plant sterol concentrations (mean±SE, µg/dl) during the home diet and after each intervention period (n=24). Vegetable oil based stanol ester margarine, TS = tall oil based stanol ester margarine.

	Home diet	VS	TS
Campestanol	47±2	58±3	47±3
Sitostanol	94±3	92±5	96±5
Campesterol	472±37	337±25	350±28
Sitosterol	277±17	198±12	227±15

* p<0.05 or less

Table 3. Mean changes (±SE) in the serum plant sterol concentrations (µg/dl), (n=24). VS = Vegetable oil based stanol ester margarine, TS = tall oil based stanol ester margarine, HD = home diet.

	Δ (VS - HD)	Δ (TS - HD)	Δ (VS - TS)
Campestanol	11±2*	0±2	11±2*
Sitostanol	-2±3	2±4	-4±4
Campesterol	-134±19*	-122±21*	-12±13
Sitosterol	-80±11*	-51±12*	-29±8*

* p<0.05 or less

Both test margarines significantly lowered serum campesterol and serum sitosterol levels. The serum concentration of campesterol is known to reflect intestinal cholesterol absorption in humans (29, 39). Thus, the lower the campesterol value, the lower the percentage of intestinal cholesterol is absorbed.

Marked falls in serum campesterol levels (25-28%) during the study periods indicates that both stanol ester margarines decreased the intestinal absorption of chol st rol. Furthermore, no differences in the serum sitostanol concentration could be seen while mean serum campestanol concentration after the vegetabl oil stanol ester period was significantly higher than at the home diet and after the

tall oil stanol ester period. However, the absolute concentration of campestanol was only about 63% of that of sitostanol, which is generally regarded as virtually unabsorbable. This low serum concentration of campestanol clearly indicates that the absorption of campestanol is very limited, which is in conflict with the results presented by Heinemann et al. (20). Thus, since stanol mixtures containing high levels of sitostanol are regarded as safe for human ingestion, stanol mixtures containing substantial amounts of campestanol must be regarded as equally safe based on the fact that campestanol is like sitostanol virtually unabsorbable.

The preparation of the stanol ester composition of the invention and the margarines used in the above clinical studies are disclosed in detail in the following working examples:

Example 1: Hydrogenation of sterol mixtures.

A commercially available sterol mixture obtained from vegetable oil distillate (composition: brassicasterol 2.7%, campesterol 26.7%, stigmasterol 18.4% sitosterol 49.1% and sitostanol 2.9%) was hydrogenated in a pilot scale reactor (25 l). 26 g of a fibrous Pd catalyst (Smop-20; Pd content 10 weight-%, Smoptech, Turku, Finland), 26 g distilled water for the activation of the catalyst and 11.7 kg propanol was feed into the reactor. The reactor was flushed with nitrogen and the activation of the catalyst was carried out under hydrogen gas at a pressure of 1 bar and at a temperature of 65°C for 30 min. After the activation the blend was cooled to 40°C, after which 1.3 kg of the sterol blend was added.

The propanol sterol mixture was heated under nitrogen atmosphere to 65°C, after which nitrogen was displaced by hydrogen. After that a thorough flushing with hydrogen was done, the hydrogenation reaction was carried out at a hydrogen pressure of 1 bar. The normal conversion time is about 120 min. The conversion can easily be monitored by taking aliquots, which are analyzed by HPLC.

The hydrogen pressure was dropped and the reactor was flushed with nitrogen. The fibrous catalyst was filtered off with nitrogen pressure. The propanol stanol blend was left to crystallize overnight at 10°C aft r which the stanol crystals were vacuum filt red and the cake was washed ith 0.5 kg cold propanol. Th obtained stanol mixture was dried at 60°C in a vacuum cupboard. Th yield was 75% and the composition of the obtained stanol mixture was as follows according to

capillary GC analysis: campesterol 0.2%, campestanol 28.9%, stigmasterol 0.1%, sitosterol 0.2%, sitostanol 70.1%. It should be noted that brassicasterol is hydrogenated into 24β-methyl cholestanol, an epimer of campestanol, but since these appear in the same peak with ordinary capillary gas chromatographic procedures which is unable to separate according to chirality, it is usually calculated as campestanol. Based on the initial sterol mixture the content of 24β-methyl cholestanol should be 2.7%.

Example 2. Preparation of stanol fatty acid esters.

A stanol fatty acid ester mixture was prepared on a pilot scale. 6 kg stanols obtained by combining several batches obtained by the hydrogenating procedure given in example 1 was dried overnight at 60°C and esterified with a 8.6 kg low erucic acid rapeseed oil methyl ester mixture. The sterol composition of the stanol blends used was as follows: Campesterol 0.4%, campestanol (+ 24β-methyl cholestanol) 29.7%, stigmasterol 0.1%, sitosterol 0.4% and sitostanol 68.0%. The stanol content of the blend was 98.2%. The esterification was carried out as follows:

A mixture of stanols and low erucic rapeseed oil fatty acid methyl ester was heated in a reactor vessel at 90-120°C under a vacuum of 5-15 mmHg. After drying for 1 hour, 21 g Na-ethylate was added and the reaction was continued for about 2 hours. The catalyst was destroyed by the addition of 30% water (by weight) at 90°C. After phase separation the water phase was removed and a second washing was carried out. After the separation of the water phase, the oily phase was vacuum dried at 95°C with a stirring effect of 200 rpm. The stanol fatty acid mixture was lightly bleached for 20 min. at 30 mmHg and a temperature of 110°C with 1.0% of bleahing earth (Tonsil Optimum FF, Südchemie, Germany) under a stirring effect of 200 rpm. The bleaching earth was filtered off and the obtained mixture of fatty acid methyl esters and stanol fatty acid esters can be added as such to fat blends prior to deodorization or the excess of methyl esters can be distilled off under vacuum. Accordingly the blend can be deodorized to obtain a tasteless stanol fatty acid ester mixture, which can be added as such to different food manufacturing processes.

The conversion of the esterification process is normally >99% measured by a fast HPLC method and the yield is in the range of 95%.

Example 3: Production of margarines for the clinical studies.

80% margarines with tall oil stanol fatty acid esters and vegetable oil based stanol fatty esters were produced on a Gerstenberg & Agger 3 x 57 pilot scale perfector. Tall oil stanol fatty acid esters were obtained from the normal production of Benecol® margarine by Raision Margariini, Finland. A normal trans fatty acid free fat blend (composition: 30% non-hydrogenated interesterified vegetable fat and 70% liquid LEAR oil) to wich the stanol fatty acid mixtures were added was used. The stanol content of the final product was targeted to be 12 g/100 g product, which would provide a daily intake of 3 g stanols at usage level of 25 g/day. The products were produced according to following recipe:

Fat blend including the stanol fatty acid esters	80 %
Water	19 %
Salt	0.5 %
Emulsifier, Dimodan BP	
Na-bicarbonate and citric acid as pH-regulating agents	
β-carotene as colouring agent	
Flavours.	

The obtained margarines were packed into 250 g polypropene tubs, which were sealed by an aluminium foil. The taste and texture of the products were equal to commercial margarines.

The stanol content of the tall oil stanol margarine was 12.7 g/100 g product and of the vegetable oil based stanol margarine 12.6 g/100 g product. The sterol composition of the two products were as follows:

	Tall oil based stanol margarine	Vegetable oil based stanol margarine
Brassicasterol	0.3%	0.4%
Campesterol	2.2%	2.4%
Campestanol	7.5%	27.6%
Sitosterol	7.4%	4.2%
Sitostanol	82.5%	63.8%
Others	0.1%	1.6%

List of Referenc s:

US Patent specification

Ref. Nr.

1 Eugster C, Eugster C, Haldemann W, Rivara G. Sterols, their fatty acid esters and glocosides; processes for their preparation; spontaneously dispersible agents containing these compounds, and their use for treatment of tumors. 1993. US Patent No 5,270,041.

2 Miettinen TA, Vanhanen H, Wester I. Use of stanol fatty acid ester for reducing serum cholesterol level. 1996. US Patent No 5,502,045.

3 Straub CD. Stanols to reduce cholesterol absorption from foods and methods of preparation and use thereof. 1993. US Patent No 5,244,887.

4 Clear cooking and salad oils having hypocholesterolemic properties. 1973. US Patent No 3,751,569.

Other patent specification

5 Baltes J, Merkle R. Verfahren zur Herstellung eines Gemisches aus pflanzlichen und tierischen Ölen bzw. Fetten und Fettsäurestemestern. German patent DE 22 48 921.

Other publications

6 Amstrong MJ, Carey MC. Thermodynamic and molecular determinants of sterol solubilities in bile salt micelles. J Lipid Res 1987; 28: 1144-1155.

7 Augustine RL, Reardon Jr. EJ 1969. The palladium catalyzed hydrogenation of cholesterol. Org Prep and Proced 1969; 1: 107-109.

ENSDOCID: <WO 9806405A1 I >

8 Becker M, Staab D, Von Bergmann K. Treatment of severe familial hypercholesterolemia in childhood with sitosterol and sitostanol. J pediatr 1993; 122: 292-296.

9 Czubayko F, Beumers B, Lammsfuss S, Lütjohann D, von Bergmann K. A simplified micro-method for quantification of fecal excretion of neutral and acidic sterols for outpatient studies in humans. J Lipid Res 1991; 32: 1861-1867.

10 Dayal B, Tnt GS, Batta AK, Speck J, Khachadurian AK, Shefer S, Salen G. Identification of 5-α stanols in patients with sitosterolemia andxanthomatosis: stereochemistry of the protonolysis of steroidal organoboranes. Steroids 1982; 40: 233-243.

11 Dutta PC, Appelqvist LÅ. Saturated sterols (stanols) in unhydrogenadted and hydrogenated edible vegetable oils and in cereal lipids. J Sci Food Agric 1996; 71: 383-391.

12 Grundy SM, Mok HYI. Effects of low dose phytosterols on cholesterol absorption in man. In: Greten H (Ed.) Lipoprotein metabolism. Springer-Verlag, Berlin, Heidelberg, New York, 1976: 112-118.

13 Gylling H, Miettinen TA, Serum cholesterol lowering by dietary sitostanol is associated with reduced absorption and synthesis of cholesterol and decreased transport of LDL apoprotein B in men with type II diabetes. In: Gotto Jr AM, Mancini M, Richter WO, Schwandt P (Eds.) Treatment of severe dyslipoproteinemia in the prevention of coronary heart disease. 4th Int Symp Munich 1992, Karger, Basel, 1993: 57-59.

14 Gylling H, Miettinen TA. Serum cholesterol and cholesterol and lipoprotein metabolism in hypercholesterolemic NIDDM patients before and during sitostanol ester-margarine treatment. Diabetologia 1994; 37: 773-780.

15 Gylling H, Siimes MA, Miettinen TA. Sitostanol ester margarine in dietary theatm nt of children with familial hyperchol sterolemia. J Lipid Res 1995; 36: 1807-1912.

16 Hassan AS, Rampone AJ. Intestinal absorption and lymphatic transport of cholesterol and ß-sitostanol in the rat. J Lipid Res 1979; 20: 646-653.

17 Heinemann T, Leiss O, von Bergmann K. Effect of low-dose sitostanol on serum cholesterol in patients with hypercholesterolemia. Atherosclerosis 1986; 61: 219-223.

18 Heinemann T, Pietruck B, Kullack-Ublick G, von Bergmann K. Comparison of sitosterol and sitostanol on inhibition of intestinal cholesterol absorption. Agents Actions (Suppl) 1988; 26: 117-122.

19 Heinemann T, Kullak-Ublick G-A, Pietruck B, von Bergmann K. Mechanisms of action of plant sterols on inhibition of cholesterol absorption. Eur J Clin Pharmacol 1991; 40: 59-63.

20 Heinemann T, Axtmann G, von Bergmann K. Comparison of intestinal absorption of cholesterol with different plant sterols in man. Eur J Clin Invest 1993; 23: 827-831.

21 Ikeda I, Sugano M. Comparison of absorption and metabolism of ß-sitosterol and ß-sitostanol in rats. Atherosclerosis 1978; 30: 227-237.

22 Ikeda I, Tanabe Y, Sugano M. Effects of sitosterol and sitostanol on micellar solubility of cholesterol. J Nutr Sci Vitaminol 1989; 35: 361-369.

23 Ikeda I, Kawasaki A, Samezima K, Sugano M. Antihypercholesterolemic activity of ß-sitostanol in rabbits. J Nutr Sci Vitaminol 1981; 27: 243-251.

24 Jandacek RJ, Webb MR, Mattson FH. Effect of an aqueous phase on the solubility of cholesterol in an oil phase. J Lipid Res 1977; 18: 203-210.

25 Lees RS, Lees AM. Effects of sitosterol therapy on plasma lipid and lipoprotein concentrations. In: Gret n H (Ed.) Lipoprotein metabolism. Springer-Verlag, Berlin, Heidelberg, New York, 1976: 119-124.

26 Lees AM, Mok HYI, Lees RS, McCluskey MA, Grundy SM. Plant sterols as cholesterol-lowering agents: clinical trials in patients with hypercholesterolemia and studies of sterol balance. Atherosclerosis 1977; 28: 325-338.

27 Ling WH, Jones PJH. Minireview dietary phytosterols: A review of metabolism, benefits and side effects. Life Sciences 1995; 57: 195-206.

28 Mattson FH, Grundy SM, Crouse JR. Optimizing the effect of plant sterols on cholesterol absorption in man. Am J Clin Nutr 1982; 35: 697-700.

29 Miettinen TA, Koivisto P. Non-cholesterol sterols and bile acid production in hypercholesterolaemic patients with ileal bypass. In: Paumgarter G, Stiehl A, Gerok W (Eds.). Bile acid and concentration in health and disease. MTP Press, Boston 1983: 183-187.

30 Miettinen TA, Vanhanen H. Dietary sitostanol related to absorption, synthesis and serum level of cholesterol in different apolipoprotein E phenotypes. Aterosclerosis 1994; 105: 217-226.

31 Miettinen TA, Puska P, Gylling H, Vanhanen H, Vartiainen E. Reduction of serum cholesterol with sitostanol-ester margarine in a mildly hypercholesterolemic population. New Engl J Med 1995; 333: 1308-1312.

32 Pollak OJ. Effect of plant sterols on serum lipids and atherosclerosis. Pharmac Ther 1985; 31: 177-208.

33 Salen G, Ahrens Jr. EH, Grundy SM. Metabolism of ß-sitosterol in man. J Clin Invest 1970, 49: 952-967. J Nutr Sci Vitaminol 1981; 27: 243-251.

34 Sugano M, Kamo F, Ikeda I, Morioka H. Lipid-lowering activity of phytostanols in rats. Atherosclerosis 1976; 24: 301-309.

35 Sugano M, Morioka H, Ikeda I. A comparison of hypocholesterolemic activity of ß-sitosterol and ß-sitostanol in rats. J Nutr 1977; 107: 2011-2019.

36 Vanhanen HT, Miettinen TA. Effects of unsaturated and saturated dietary plant sterols on their serum contents. Clin Chim Acta 1992; 205: 97-107.

37 Vanhanen HT, Blomqvist S, Enholm C, Hyvönen M, Jauhiainen M, Torstila I, Miettinen TA. Serum cholesterol, cholesterol precursors, and plant sterols in hypercholesterolemic subjects with different apoE phenotypes during dietary sitostanol ester treatment. J Lipid Res 1993; 34: 1535-1544.

38 Vanhanen HT, Kajander J, Lehtovirta H, Miettinen TA. Serum levels, absorption efficiency, faecal elimination and synthesis of cholesterol during increasing doses of dietary sitostanol esters in hypercholesterolaemic subjects. Clin Sci 1994; 87: 61-67.

39 Tilvis RS, Miettinen TA. Serum plant sterols and their relation to cholesterol absorption. Am J Clin Nutr 1986; 43: 92-97.

What is claimed is:

1. A composition of plant stanols for use as a serum cholesterol level lowering substance and comprising sitostanol, the composition further comprising at least 10% campestanol obtained by hydrogenation of a phytostanol mixture.

2. The composition of Claim 1, comprising from 20% to 40%, preferably from 25% to 35% campestanol.

3. The composition of Claim 1 or 2, comprising from 50% to 80% of sitostanol.

4. A composition of plant stanol fatty acid esters comprising a sitostanol fatty acid ester for use as a serum cholesterol level lowering substance, the composition further comprising at least 10% of a campestanol fatty acid ester.

5. The composition of Claim 4, comprising from 20% to 40%, preferably from 25% to 35%, e.g. about 30% of the campestanol fatty acid ester.

6. The composition of Claim 4 or 5, comprising from 50% to 80% of the sitostanol fatty acid ester.

7. The use of a composition of any of Claims 1 to 6 as such or as part of the diet, e.g. in fat-containing foods, to be consumed for lowering serum cholesterol levels.

8. A food substance containing a plant stanol composition comprising sitostanol or a fatty acid ester thereof effective in lowering serum cholesterol levels, the composition further comprising a substantial amount of campestanol or a fatty acid ester thereof such that the weight ratio of campestanol or its fatty acid ester to sitostanol or its fatty acid ester is from 1:9 to 4:6.

9. The food substance of Claim 8, wherein said weight ratio is from 2:8 to 3.5 : 6.5.

10. Th us of a phytost rol mixture, comprising in addition to sitost rol a substantial amount of campesterol, as a raw material for producing a serum cholesterol level lowering composition or food substance according to any of Claims 1 to 6, 8 and 9.

AMENDED CLAIMS

[received by the International Bureau on 15 July 1997 (15.07.97); original claim 1 amended; remaining claims unchanged (2 pages)]

1. A composition of plant stanols for use as a serum cholesterol level lowering substance and comprising sitostanol, the composition further comprising at least 10% campestanol.

2. The composition of Claim 1, comprising from 20% to 40%, preferably from 25% to 35% campestanol.

3. The composition of Claim 1 or 2, comprising from 50% to 80% of sitostanol.

4. A composition of plant stanol fatty acid esters comprising a sitostanol fatty acid ester for use as a serum cholesterol level lowering substance, the composition further comprising at least 10% of a campestanol fatty acid ester.

5. The composition of Claim 4, comprising from 20% to 40%, preferably from 25% to 35%, e.g. about 30% of the campestanol fatty acid ester.

6. The composition of Claim 4 or 5, comprising from 50% to 80% of the sitostanol fatty acid ester.

7. The use of a composition of any of Claims 1 to 6 as such or as part of the diet, e.g. in fat-containing foods, to be consumed for lowering serum cholesterol levels.

8. A food substance containing a plant stanol composition comprising sitostanol or a fatty acid ester thereof effective in lowering serum cholesterol levels, the composition further comprising a substantial amount of campestanol or a fatty acid ester thereof such that the weight ratio of campestanol or its fatty acid ester to sitostanol or its fatty acid ester is from 1:9 to 4:6.

9. Th food substanc of Claim 8, wher in said weight ratio is from 2:8 to 3.5 : 6.5.

10. The us of a phytosterol mixture, comprising in addition to sitosterol a substantial amount of campesterol, as a raw material for producing a s rum

cholesterol l vel low ring composition or food substance according to any of Claims 1 to 6, 8 and 9.

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A. CLASSIFICATION OF SUBJECT MATTER

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B. FIELDS SEARCHED

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SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CASONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Atherosclerosis, Volume 24, 1976, Michihiro Sugano et al, "Lipid-Lowering Activity of Phytostanols in Rats" page 301 - page 309	1-10
A	US 5502045 A (TATU MIETTINEN ET AL), 26 March 1996 (26.03.96)	1-10
A	US 5244887 A (CARL D. STRAUB), 14 Sept 1993 (14.09.93)	1-10

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

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US 5502045 A	26/03/96	AU 664827 B	07/12/95
		CA 2102112 A	04/11/92
		EP 0594612 A	04/05/94
		FI 934869 D	00/00/00
		FI 964951 A	11/12/96
		JP 6506909 T	04/08/94
		NO 933966 A	02/11/93
		PL 166991 B	31/07/95
		WO 9219640 A	12/11/92
US 5244887 A	14/09/93	NONE	

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Tab. 1.: Fettsäure-Zusammensetzung wichtiger pflanzlicher Fette (Anzahl C-Atome: Anzahl Doppelbindungen).

Fett	10:0	12:0	14:0	16:0	18:0	18:1	18:2	18:3	20:1	22:1
Rapsöl (neu)	–	–	0,5	4	1	60	20	9	2	2
*Sonnenblumenöl	–	–	–	6	4	28	61	–	–	–
Leinöl	–	–	–	5	4	22	17	52	–	–
*Kokosfett	7	48	17	9	2	7	1	–	–	–
Palmkernfett	5	50	15	7	2	15	1	–	–	–
*Palmöl	–	–	2	42	5	41	10	–	–	–
*Sojaöl	–	–	–	10	5	21	53	8	0,5	–
*Erdnußöl	–	–	–	10	3	41	36	–	1	–

Nachweis der Fetthärtung sowie Nachweis von Zusätzen (Antioxidantien) bzw. Fremdbestandteilen (techn. Öle, Pestizide u. a.). Der Gesamtfett-Gehalt eines Lebensmittels wird meist durch gravimetr. Analyse des Ether- bzw. Petrolether-Extraktes ermittelt sowie schneller anhand der ^{1}H-NMR-Spektroskopie. Zur Charakterisierung von F. werden in der klass. Fettchemie verschiedene Kennzahlen (vgl. Fettkennzahlen) verwendet, die heute allerdings durch chromatograph. Verf. z. T. überholt sind. Die Bestimmung der Fettsäure-Zusammensetzung in den Triacylglyceriden erfolgt meist gaschromatograph. nach Freisetzung der Acyl-Reste als Methylester (Umesterung mit Na-methylat). In bestimmten Fällen können einzelne Fettsäuren als Indikatoren für die Fettart herangezogen werden (Tab. 2). So kann z. B. anhand von *Behensäure (22:0) Erdnußöl nachgewiesen werden. Die Unterscheidung von *cis*- u. *trans*-Fettsäuren (Nachweis der Fetthärtung bei pflanzlichen Fetten) erfolgt heute durch Kapillargaschromatographie. Eine Anreicherung von analyt. interessanten Neben-Fettsäuren wird mittels spezieller Trenntechniken durchgeführt, z. B. der Argentationschromatographie (Trennung der geometr. Isomere) od. über *Harnstoff-Addukte (Methyl-verzweigte Fettsäuren: Nachweis von Seetierölen in Pflanzenfetten). Aussagekräftiger ist allerdings häufig das Triacylglycerid-Muster, das sowohl durch direkte gaschromatograph. Analyse (vgl. Kakaobutter) als auch durch HPLC ermittelt werden kann. Auch die Bestimmung der Zusammensetzung des *Unverseifbaren ermöglicht die Ermittlung der verwendeten Fettart (Tab. 3). Zur Bestimmung des Gehaltes an freien *Fettsäuren, die die Fettqualität beeinflussen, dient die Säurezahl (vgl. Fettkennzahlen). Der Oxid.-Zustand der F. kann über die Peroxid-Konz. (vgl. Peroxid-Zahl) sowie den Gehalt an Carbonyl-Verb. (vgl. Thiobarbitursäure-Test), die aus der *Autoxidation hervorgehen, bewertet werden. Eine geeignetere Meth. zur Bewertung der Fettqualität ist jedoch die selektive quant. Erfassung der Aroma-wirksamen Verbindungen[2]. Voraussagen zur Lagerstabilität von F. erlaubt der *Swift-Test.

– *E* fats and oils – *F* graisses et huiles – *I* grassi e oli – *S* grasas y aceites

Lit.: [1] J. Am. Oil Chem. Soc. 65, 702 (1988). [2] Lebensm. Wiss. Technol. 23, 59 f. (1990).

allg.: Belitz-Grosch (4.), S. 600 ff. ▪ Christie, HPLC and Lipids, Oxford: Pergamon Press 1987 ▪ Fat Sci. Technol. 93, 526–535 (1991); 94, 477–489, 490–493, 494 f., 558–560 (1992) ▪ Perkins, Analysis of Fats, Oils and Lipoproteins, Illinois: Am. Oil Chem. Soc. 1991. – *[Z 15.01–15.04, 15.70, 15.80, 15.10]*

Tab. 2: Fettsäuren als Indikatoren für Fettarten.

Fettsäure	Anteil[a] (%)	Indikator für
4:0	3,7	Milchfett
12:0	45	Kokos-, Palmkern- und Babassufett
18:1 (9)	65–85[b]	Teesamen-, Oliven- u. Haselnußöl
18:3 (9, 12, 15)	9	Soja, Raps- u. Rüböl (auch erucasäurefrei)
18:2 (9, 12)	50–70[b]	Sonnenblumen-, Maiskeim-, Baumwollsaat-, Weizenkeim- u. Sojaöl
22:0	3	Erdnußöl
20:4 (5, 8, 11, 14)	0,1–0,6	Tierische Fette
18:1 (9, 12-OH)	80	Ricinusöl
trans-Fettsäuren		Partiell od. vollständig hydrierte Fette[c]
Methyl-verzweigte Fettsäuren	0,2–1,6	tierische Fette[d]

[a] Der Anteil an der Fettsäure-Zusammensetzung ist als Mittelwert angegeben, wenn nur ein Wert notiert ist.
[b] Die hohe Konz. dieser Fettsäure ist charakteristisch.
[c] Zu beachten ist, daß tier. Fette bis zu 10 % *trans*-Fettsäuren enthalten können.
[d] Bes. hoch in den Seetierölen (um 1 %).

Tab. 3: Identifizierung von Fettarten über die Analyse von Bestandteilen des „Unverseifbaren".

Analyse	Nachweis
Squalen	Olivenöl, Reisöl, Fischleberöl
Campesterin/Stigmasterin[a]	Kakaobutter-Austauschfette
Cholesterin[c]	Tierische Fette
Carotine	Rohes Palmöl
γ-/β-Tocopherol[b]	Maisöl
β-Tocopherol	Weizenkeimöl
α-/γ-Tocopherol[b]	Sonnenblumenöl
γ-/δ-Tocopherol[b]	Sojaöl
Alkoxylipide	Schweinefett/Rinderfett

[a] Konz.-Verhältnis charakteristisch.
[b] Konz. der einzelnen Verb. u. Konz.-Verhältnis charakteristisch.
[c] Cholesterin-Konz. muß > 5 % der Sterin-Fraktion sein.

Fettfisch. Bez. für *Fische mit einem Fettgehalt von über 10% (s. a. Magerfische u. Seetieröle). Zu den

Table 2: Fatty acids as indicators for types of fat

Fatty Acid	Portion[a] (%)	Indicator for
4:0	3.7	milk fat
12:0	45	coconut, palm nut and babassu fat
18:1 (9)	65-85[b]	tea seed, olive and hazelnut oil
18.3 (9, 12, 15)	9	soybean, rape, colza oil (also erucic acid-free)
18:2 (9, 12)	50-70[b]	sunflower, corn, cotton seed, wheat germ and soybean oil
22:0	3	peanut oil
20:4 (5, 8, 11, 14)	0.1-0.6	animal fats
18:1 (9, 12-OH)	80	castor oil
trans-fatty acids		partially or completely hydrogenated fats[c]
methyl-branched fatty acids	0.2-1.6	animal fats[4]

[a] The portion of the fatty acid composition is given as an average if only one value is noted.

[b] The high concentration of this fatty acid is characteristic.

[c] It should be noted that animal fats can contain up to 10% of *trans*-fatty acids.

[d] Especially high in sea animal oils (by 1%).

Table 3: Identification of types of fat by analysis of components of the "unsaponifiables".

Analysis	Detection
squalene	olive oil, rice oil, cod-liver oil
campesterol/stigmasterol[a]	cocoa butter substitute fats
cholesterol[c]	animal fats
carotenes	raw palm oil
γ-/β tocopherol[b]	corn oil
β-tocopherol[b]	wheat germ oil
α-/γ-tocopherol[b]	sunflower oil
γ-/δ-tocopherol[b]	soybean oil
alkoxylipids	lard/beef suet

a Concentration ratio is characteristic.

b Concentration of the individual compound and concentration ratio is characteristic.

c Cholesterol concentration must be >5% of the sterol fraction.